Enzymic Mechanism of Starch Synthesis in Ripening Rice Grains

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It has long been believed that phosphorylase is involved in the synthesis of starch, which constitutes 70 to 80% of the mature rice grain. Aimi and his associates (1, 2, 3) found that during the ripening process a parallel relationship exists between starch synthetase and phosphorylase activity in rice grains. But we can not exclude the possible existence of mechanisms other than phosphorylase reaction for starch formation. The high ratio of inorganic phosphate to sugar phosphate in plants, which is unfavorable to starch formation (1, 10, 31), raises doubts about the role of phosphorylase in polysaccharide synthesis.

An important development in the field of polysaccharide biochemistry has been the recent discovery of starch synthetase by Leloir's group (8, 14), which proposed the following enzymic reaction:

$$\text{UDPG + acceptor (G)}_n \rightarrow \text{UDP + starch (G)}_{n+1}.$$

Many workers have accepted this view (23, 27) and in contrast, have relegated the role of phosphorylase to the breakdown of polysaccharides (8, 36).

In some plants, including rice, sucrose is synthesized in the leaves and stems, and is translocated to the grains where it eventually is transformed into starch (12, 25, 26, 35). In assigning starch synthetase to its role in starch synthesis, the connection between this enzymic mechanism and sucrose metabolism should be recognized. Enzymic evidence pertaining to sucrose-starch interconversion has not been reported, but since sucrose synthesis is catalyzed by sucrose synthetase (6, 22), the possible existence of a link between the 2 reactions involving UDPG is worth exploration. The present investigation is primarily concerned with the study of starch synthetase in ripening rice grains, and, secondly, with experiments on sucrose synthetase as a possible clue to the mechanisms of sucrose-starch interconversion.

Materials and Methods

Growing and Sampling of Rice Plants. Peta, an indica variety, and Chianung 242, a japonica variety, were used. Plants were grown in soil in a greenhouse. Peta and Chianung 242 flowered 4 months and 3 months, respectively, after planting. Panicles at the same developmental stage were taken from different pots. The grains were stripped off the panicles, mixed thoroughly, and a subsample removed for analysis of sugar components in the grains. For the enzymic study, panicles with grains in the midmilk stage (usually 10–15 days after pollination) were chosen.

Sugar Analyses. A certain amount of fresh whole rice grains including seedcoat was first heated at 90°C for 30 minutes, then dried at 60°C for 4 hours and the dry weight determined. The dried grains were ground with a Wiley mill (40 mesh). Usually, a 3-g sample of the powdered grain was continually extracted with 95% ethanol, and the residual matter (starch) was hydrolyzed with 2% HCl for glucose analysis. The ethanol extract was concentrated in a flash evaporator, treated with lead acetate, and finally deionized with a small amount of Amberlite IR-120 and IR-4B ion-exchange resins. It was then made up to a 20-ml volume, and aliquots were analyzed for the total sugar content (9) and for reducing sugars (33). The amount of nonreducing sugar was calculated from the difference of the above 2 values. For further column chromatographic analyses of sugar components, the method of Mori and Nakamura (18) was employed.

Enzymic Studies

Starch Synthetase. This enzyme was prepared using 15 g of the immature rice grains (14). The average total nitrogen content was 7 to 8 μg per milligram of starch for the 2 varieties. Total and protein nitrogen were determined by nesslerization. Starch granules prepared in this manner were kept in a deep freeze (−20°C) without much loss of activity over a 2 month period. The enzyme assay was a modified method of Leloir et al. (14); a standard incubation mixture contained 6 μmoles of glycine buffer (pH 8.4), 0.2 μmole of EDTA, 0.3 μmole of UDPG, 0.4 μmole of glucose-6-P (when added) in a total volume of 17 μl and with stated amounts of starch granules. The mixture was then incubated at 37°C for varying periods of time. For longer incubation periods, the test tubes were stoppered to prevent evaporation. After the incubation, the UDP formed was spectrophotometrically assayed (520 mμ) by the pyruvic
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isotope experiments, UDP-glucose-C\textsuperscript{14} \ was diluted twofold with a 6 x 10\textsuperscript{-2} M UDPG solution, and 0.18 to 0.24 \mu\textit{mole} was added to each test tube. After the incubation, the transfer of glucose-C\textsuperscript{14} to starch was determined according to the method of Leloir et al. (14). The reaction system for the glucose-C\textsuperscript{14} transfer from sucrose-C\textsuperscript{14} to starch contained: 4 \mu\textit{moles} of glycine buffer (pH 8.4), 0.1 \mu\textit{mole} of EDTA, 0.3 \mu\textit{mole} of MgSO\textsubscript{4}, 0.36 \mu\textit{mole} of UDP, 5 \mu\textit{moles} of sucrose-C\textsuperscript{14} (2.28 x 10\textsuperscript{6} cpm), 5 \mu\textit{L} of sucrose synthetase (fraction II, see below), and 6 mg of starch granules in a total volume of 17 \mu\textit{L}. Changes in the composition of this reaction mixture are indicated in the table. Incubation was for 3 hours. To insure complete solubilization of the sample, a heated starch solution was further hydrolyzed with dilute HCl. 10 ml of scintillator (28) was added to 0.1 ml aliquots of the sample contained in vials and radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer.

Sucrose Synthetase. In preparing the enzyme (14) usually 15 g of rice grains were ground with 40 ml of 0.05 M potassium phosphate buffer (pH 7.0). The extract squeezed through cheese cloth was spun down at 15,500 rpm for 15 minutes to obtain the supernatant (fraction I). A fraction precipitable by 0.2 to 0.6 saturation of (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was dissolved in 10 ml of distilled water and dialyzed against water for several hours in a cold room. It was then centrifuged to get a clear supernatant fluid (fraction II) which was stable for about a week in the refrigerator (1\textdegree). A standard reaction mixture contained 100 \mu\textit{moles} of Tris buffer (pH 7.4), 3 \mu\textit{moles} of MgSO\textsubscript{4}, 5 \mu\textit{moles} of fructose, 0.02 to 0.1 ml of an appropriate enzyme preparation, and a final addition of 1.2 \mu\textit{moles} of UDPG in a total volume of 0.6 ml after 10 minutes preincubation at 37\textdegree. Sucrose formation was determined by the resorcinol method of Roe (30).

UDPG-pyrophosphorylase and UDP-phosphokinase. Fraction II obtained above was used to examine the activity of these 2 enzyme systems. Instead of determining UDPG formation, the reaction was coupled to the sucrose synthesizing enzyme and sucrose formation was determined. The assay mixture was identical to that of the sucrose synthetase system, except that UDPG was substituted by 1 \mu\textit{mole} of UTP and 2 \mu\textit{moles} of glucose-1-P for the pyrophosphorylase and 2 \mu\textit{moles} of UDP and 5 \mu\textit{moles} of ATP for the kinase systems.

Hexokinase. The preparation of the enzyme was based on the method of Saltman (32): 70 g of rice grains were ground in a mortar with 50 ml of 0.1 M Tris buffer (pH 7.5), the extract squeezed through cheese cloth, and spun down at 9,200 rpm for 5 minutes. The supernatant fluid was again centrifuged at 15,500 rpm for 20 minutes to get a crude extract, fraction A. The latter fraction was treated with 1 % acetic acid to adjust the pH to 5.5 and the precipitate was removed by centrifugation. The pH of the supernatant fluid was adjusted to 7.5 with 1 x NaOH (fraction B). Fraction B was treated with solid (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and the fraction precipitable at 0.5 saturation (fraction C) and 0.5 to 0.6 saturation (fraction D) were separated by centrifugation. Each was dissolved in 0.1 M Tris buffer (pH 7.5). Before the enzymic assay, each fraction was dialyzed against water for 4 to 6 hours. The reaction mixture contained 100 \mu\textit{moles} of Tris buffer (pH 7.5), 10 \mu\textit{moles} of MgSO\textsubscript{4}, 5 \mu\textit{moles} of glucose or fructose, 10 \mu\textit{moles} of ATP, 50 \mu\textit{moles} of NaF and 0.2 ml of the enzyme preparation in a total volume of 1.0 ml. The samples were incubated for 30 minutes at 30\textdegree, and 1.0 ml each of 0.3 x Ba (OH)\textsubscript{2} and 5% ZnSO\textsubscript{4} were added to remove the esterified sugar. The residual free hexoses were determined by an appropriate method (30, 33).

Oxidative Phosphorylation. The particulate fraction was prepared from 50 g of rice grains, using the method for obtaining mitochondria from sweet potato roots (4). The final precipitate was suspended in 2.5 ml of the medium. The assay mixture contained 12.5 \mu\textit{moles} of potassium phosphate, 20 \mu\textit{moles} of \alpha-\textit{ketoglutarate}, 2.5 \mu\textit{moles} of ATP, 1 \mu\textit{mole} of DPN, 0.5 mg of thiamine pyrophosphate, 10 \mu\textit{moles} of MgCl\textsubscript{2}, 20 \mu\textit{moles} of glucose, 100 units of crystalline hexokinase, and 0.5 ml of the particulate suspension in a final volume of 1.5 ml. Incubation was at 30\textdegree for 45 minutes and P\textsubscript{i} disappearance was measured by Nakamura's method (21).

Ion-exchange Chromatographic Separation of Sugar Phosphates. The following reaction mixtures were employed: hexokinase system—100 \mu\textit{moles} of Tris buffer (pH 7.4), 30 \mu\textit{moles} of MgSO\textsubscript{4}, 30 \mu\textit{moles} of fructose-C\textsuperscript{14} (2.24 x 10\textsuperscript{6} cpm), 60 \mu\textit{moles} of ATP, 50 \mu\textit{moles} of NaF, and 0.4 ml of enzyme preparation (fraction D as described above), in a total volume of 1.8 ml; and sucrose utilization system—100 \mu\textit{moles} of Tris buffer (pH 7.4), 30 \mu\textit{moles} of MgSO\textsubscript{4}, 30 \mu\textit{moles} of sucrose-C\textsuperscript{14} (1.37 x 10\textsuperscript{6} cpm), 20 \mu\textit{moles} of ATP, 12 \mu\textit{moles} of UDP, 50 \mu\textit{moles} of NaF, and 0.4 ml of the enzyme preparation in a total volume of 1.6 ml. At the end of incubation, the entire reaction mixture was heated in a boiling water bath for 4 minutes, and later deionized by adding a small amount of Amberlite ion exchange resins, IR-120 and IR-4B, and centrifuged. The clear centrifuge was subjected to ion exchange column chromatography for sugar phosphates, using Dowex-1 resin [AG 1 -x8 (200–400 mesh, chloride form) Calbiochem.] originally developed by Khy and Cohn (13) and described by Morii et al. (19). About 5 mg each of glucose-1-P and glucose-6-P were added as carriers. An authentic sample of fructose-6-P was not available for use as a carrier. This sugar phosphate, therefore, was identified from its elution from the column, as quoted in the literature. Forty milliliters of effluent was collected and an aliquot was analyzed for sugar content by the method of Dubois et al. (9) and radioactivity. The method of Roe
CPO acid, pyruvic components glucose-6-P, phosphoenolpyruvic grain (variety Peta) during ripening.

Rice of the University hexokinase and fructose-6-P. respectively. sucrose-C'4. chemical Research: chased from starch and Company, respectively. UDP-glucose-C14.
Table 1
Effect of Iodoacetamide on UDPG-starch Transglucosylase

<table>
<thead>
<tr>
<th>Iodoacetamide concentration</th>
<th>Incubation min</th>
<th>UDP formed μmole</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 × 10^{-3}M</td>
<td>60</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>86.4</td>
</tr>
</tbody>
</table>

indicating the presence of an SH-functional group. Under the same reaction conditions, this inhibitor did not affect the pyruvic phosphokinase system for UDP determination. Glucose-6-P did not stimulate markedly the starch synthetase system, although this sugar phosphate was reported to be essential in the glycogen synthetase system (15, 16).

The results of the isotope experiments eliminated the possibility of UDP formation by simple hydrolytic cleavage of the UDPG molecule catalyzed by the starch granules. Vigorous incorporation of glucose-C^{14} into the pre-existing starch molecules did occur, and this was taken as conclusive evidence of enzymic transglucosylation. The possible contamination by oligosaccharides formed by the transfer of glucose-C^{14} also was eliminated since the starch samples had been washed thoroughly.

Table II
Glucose Transfer from UDP-glucose-C^{14} (a) and Sucrose-C^{14} (b) to Amylose and Amylopectin

4.0 mg of starch granules prepared from Chianung 242 were added to each standard reaction mixture. Each of UDP-glucose-C^{14} and sucrose-C^{14} added were 0.24 μmole (3.31 × 10^4 cpm) and 7 μmole (6.84 × 10^5 cpm) respectively.

(a)

<table>
<thead>
<tr>
<th>Incubation hr</th>
<th>Starch fractions</th>
<th>Total C^{14} cpm</th>
<th>Glucose content μmole</th>
<th>Specific activity cpm/μmole</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Amylose</td>
<td>3,530</td>
<td>5.9</td>
<td>598</td>
</tr>
<tr>
<td></td>
<td>Amylopectin</td>
<td>6,400</td>
<td>18.7</td>
<td>342</td>
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<td>5</td>
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(b)

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<td>6.5</td>
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<td>Amylopectin</td>
<td>6,100</td>
<td>21.9</td>
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FIG. 4. Reaction course of starch synthetase. Starch granules (6 mg.) (see legend in fig 3) were added to the standard reaction mixture containing 0.24 μmole of UDPG. Arrow indicates the maximum level of the reaction.

To determine whether glucose was incorporated into both amylose and amylopectin after incubation with UDP-glucose-C^{14}, the starch granules were fractionated into these 2 components following the method of Leloir et al. (14), and the radioactivity was determined in each fraction. Both amylose and amylopectin incorporated glucose-C^{14} after 2 to 10 hours incubation, and the specific radioactivity in the amylose fraction was consistently higher than that in the amylopectin fraction (table IIa).

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</table>
Table III

Isolation of Sucrose Synthetase from Rice Grains

The enzyme was prepared from Chianung 242. One enzyme unit is defined as the amount of enzyme synthesizing 1 μmole of sucrose in 10 minutes under the given conditions.

<table>
<thead>
<tr>
<th>Enzyme fraction</th>
<th>Volume ml</th>
<th>Activity units/ml</th>
<th>Total amt. units</th>
<th>Protein-N mg/ml</th>
<th>Specific activity units/mg protein-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>42.0</td>
<td>10.3</td>
<td>432.6</td>
<td>0.17</td>
<td>60.0</td>
</tr>
<tr>
<td>II</td>
<td>11.8</td>
<td>18.4</td>
<td>217.1</td>
<td>0.15</td>
<td>122.5</td>
</tr>
</tbody>
</table>

Sucrose Synthetase and Sucrose-starch Interconversion. A constant supply of UDPG must somehow be provided for the starch synthesis to proceed via starch synthetase. As a first step towards solving this problem, and considering the large amounts of sucrose present in ripening rice seeds (fig 1, 2), the system involving sucrose synthetase was investigated as a possible source of UDPG. The crude extract as well as the partially purified preparation (fractions I, II) exhibited strong enzyme activities (table III). In a complete reaction system, fraction I enzyme produced 0.52 μmole and 0.84 μmole of sucrose after 10 minutes and 30 minutes incubation, respectively; negligible amounts of sucrose were formed when either UDPG or fructose was omitted or when boiled enzyme was used. The apparent equilibrium constant of the reaction \[ K'_{eq} = \frac{\text{(UDP)} (\text{sucrose}) \text{/(UDPG)} (\text{fructose})} {\text{(fructose)} (\text{UDPG})} \] was calculated to be about 2.0, an average value from 3 experiments. Thus, under the present experimental conditions, the equilibrium is in favor of sucrose synthesis instead of UDPG formation. However, the reversal of sucrose synthetase may be energetically possible when coupled with starch synthetase (I), with UDPG as an intermediate and sucrose serving as the glucose donor (II).

Sucrose + acceptor \((G)_n \rightarrow \text{fructose} + \text{starch} (G)_{n+1}\) II

The results shown in table IV indicate that in the presence of sucrose synthetase, a roughly proportional increase of glucose transfer from sucrose-C\(^{14}\) to starch was observed with increasing amounts of starch granules (experiment 1-4). The increase in the rate of glucose-C\(^{14}\) transfer as a function of UDPG concentration is shown also (experiment 5-7). There was very little incorporation of glucose-C\(^{14}\) without UDPG, whereas a marked stimulating effect of UDPG on the glucose transfer was noted. Thus, the experimental results tend to verify the reversal of sucrose synthesis as a generator of UDPG necessary for starch formation. The percentage utilization of sucrose-C\(^{14}\) as a glucose donor was rather low in each case, however. As will be seen in the table (experiment 8-9), ATP was found to inhibit rather than stimulate the glucose-C\(^{14}\) transfer from sucrose.

Provided sucrose functions truly as a glucose donor in starch formation, the pattern of C\(^{14}\) distribution of each fraction of amylose and amylopectin should be identical to that from UDP-glucose-C\(^{14}\). The results of the radioactivity determinations in the 2 fractions are shown in table IIb. The pattern is similar to the case of glucose transfer from UDP-glucose-C\(^{14}\).

Another possibility for the synthesis of UDPG may be by the UDPG pyrophosphorylase reaction (24, 34), and the coupling of this system with UDP phosphokinase to get the over-all reaction III.

\[ \text{UDP} + \text{ATP} + \text{glucose-1-P} \rightarrow \text{UDPG} + \text{ADP} + \text{PP}_1. \] III

Instead of measuring UDPG formation, both these reactions were tested for by coupling each one with sucrose synthetase, using fraction II enzyme. A

Table IV

Glucose Transfer from Sucrose-C\(^{14}\) to Starch under Various Conditions

Experiments 1 through 9 were carried out simultaneously and in duplicate; experiment 3 served as the standard system, in which the reaction mixture described in the text were employed. Sucrose-C\(^{14}\) added was 2.28 \( \times \) 10\(^5\) cpm. Starch granules were prepared from Peta.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Starch granules mg</th>
<th>UDP mmoles</th>
<th>ATP mmoles</th>
<th>Sucrose-synthetase</th>
<th>C(^{14}) in starch cpm</th>
<th>Incorporation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>360</td>
<td>—</td>
<td>+</td>
<td>1,070</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>1,290</td>
<td>0.57</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>240</td>
<td>—</td>
<td>+</td>
<td>2,440</td>
<td>1.07</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>720</td>
<td>—</td>
<td>+</td>
<td>3,060</td>
<td>1.34</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>980</td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>240</td>
<td>—</td>
<td>+</td>
<td>1,720</td>
<td>0.75</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>720</td>
<td>—</td>
<td>+</td>
<td>4,280</td>
<td>1.88</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>360</td>
<td>0.2</td>
<td>+</td>
<td>2,080</td>
<td>0.91</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>1.0</td>
<td>1.0</td>
<td>+</td>
<td>1,390</td>
<td>0.61</td>
</tr>
</tbody>
</table>
measurable amount of sucrose is formed in the complete system (fig 5A, 5B). Omission of either one of the reaction components resulted in little sucrose formation. Nakamura (22) made similar observations, using partially purified sucrose synthesizing enzyme from beans.

**Hexokinase and Oxidative Phosphorylation.** To determine the fate of free glucose and fructose, the phosphorylation of these sugars was examined. Of the various preparations, the \((\text{NH}_4)_2\text{SO}_4\) precipitable fraction at 0.5 to 0.6 saturation (fraction D) exhibited a strong hexokinase activity to both sugars (table V). The ratios of the activities of the 2 sugars (fructose/glucose) were somewhat consistent in subsequent purification steps, but it is not certain whether there is a fructose-specific kinase in rice grains. Fructokinase was isolated from peas (17).

The energy required for the hexokinase reaction may possibly be generated from the particulate components (mitochondria) of the grain cells through oxidative phosphorylation. The particulate fraction isolated from rice grain was tested for the presence of the latter system, and P/O ratios of 0.85 and 1.32 were observed in 2 separate experiments. However, both oxidative and phosphorylative activities obtained were rather weak, as compared to other plant mitochondria.

It was of interest to verify the enzymic transformation of fructose to glucose-1-P, which may establish the over-all process of sucrose-starch conversion as reaction IV, because the fructose moiety of reaction II will eventually be converted to UDPG by reaction III.

\[
\text{Sucrose} + 2 \text{ ATP} + 2 \text{ acceptor (G)}_n \rightarrow 2 \text{ starch (G)}_{n+1} + 2 \text{ ADP} + \text{PP}_i. \quad \text{IV}
\]

From our basic knowledge of the carbohydrate metabolism, it is highly predictable that the glycolytic pathway exists in rice seeds, and in fact, Dowex-1 ion-exchange column chromatographic separation of the reaction products of the fructose-C\(^4\) phosphorylation system described above showed the formation of fructose-6-P, glucose-6-P, and glucose-1-P. Similarly, formation of such hexose phosphates was substantiated by incubating sucrose-C\(^4\) in the presence of UDP and ATP, supporting the presumption of the presence of glycolytic enzymes transforming the fructose moiety derived from sucrose to glucose-1-P.

![Figure 5](https://example.com/figure5.png)

**Fig. 5.** (A) Coupling of UDPG-pyrophosphorylase with sucrose synthetase. A twofold dilution (0.5 ml) of fraction II described in table III was used as the enzyme. Open circles are for the complete system. GIP represents glucose-1-P.

(B) Coupling of UDPG-pyrophosphorylase and UDPkinase with sucrose synthetase. A twofold dilution (0.1 ml) of fraction II described in table III was used as the enzyme. Other experimental conditions were same as in (A). Open circles are for the complete system. In (A) and (B) arrows indicate the maximum level of the reaction.

**Discussion**

The net content of starch per grain is much higher than that of sucrose and the other 2 hexoses (fig 1, 2). This over-all trend indicates sucrose utilization and its accumulation in grains during the enzymic transformation to starch. Assuming sucrose to be a direct precursor in starch formation, a possible enzymic mechanism governing the over-all interconversion would be reaction II. This enzymic linking was proposed by De Fekete et al. (8), and our experimental results (table IV) tend to verify that such a mechanism operates in ripening rice grains. The question will be raised, however, whether sucrose

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**Table V**

*Isolation of Hexokinase from Rice Grains*

The enzyme was prepared from Chianung 242. One enzyme unit is defined as the amount of enzyme synthesizing 1\(\mu\)mole of hexose phosphate in 30 minutes under the given condition.

<table>
<thead>
<tr>
<th>Enzyme fraction</th>
<th>Activity units/ml</th>
<th>Protein-N mg/ml</th>
<th>Specific activity units/mg protein-N</th>
<th>Ratio fructose/glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glucose</td>
<td>fructose</td>
<td>glucose</td>
<td>fructose</td>
</tr>
<tr>
<td>A</td>
<td>8.45</td>
<td>5.20</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6.75</td>
<td>5.00</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.60</td>
<td>4.65</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>10.30</td>
<td>9.45</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 6. Proposed enzymic mechanism of starch biosynthesis. F6P, G6P, and GIP represent fructose-6-P, glucose-6-P, and glucose-1-P, respectively.

forms UDPG by the direct transglucosylation in such a reaction system. Distribution of sucrose is ubiquitous in the plant kingdom, and the enzyme may be engaged in the metabolic breakdown or utilization of sucrose, but the activity was found to be weak in the rice grain extract. Also if the entire process involves the mechanism:
sucrose → glucose + fructose → hexose phosphate → UDPG → starch

ATP should enhance the glucose transfer from sucrose to starch, because of the requirement of hexose phosphorylation. However, ATP was in fact found to be inhibitory rather than stimulative (c.f. experiment 8,9, table IV). By the isotope experiments it was found that UDPG was synthesized from sucrose only in the presence of UDP (Paper submitted to Arch. Biochem. Biophys.), substantiating the mechanism written as reaction II. Thus, by taking into account the experimental evidences in hand, the consequent over-all reaction mechanism of sucrose-starch conversion will be reaction IV and may be diagrammatically illustrated as figure 6. The validity of this view requires more study. For example, Recondo and Leloir (29) and Frydman (11) found that ADPG is much more active than UDPG as a glucose donor of starch synthetase, while UDPG is the dominant substrate for the sucrose synthesis (7). We have also isolated ADPG from ripening rice grains independently and its more efficient utilization in starch formation was confirmed (20). The inefficient utilization of sucrose-C14 in starch synthesis in vitro may be a reflection of the more important role of the ADPG pathway in the system, and further work is needed to elucidate the interconnection of this new pathway with the presently investigated UDPG system. Another reason for the poor utilization of sucrose-C14 in starch synthesis in vitro may result from difficulties inherent in reconstituting such a system. As has been noted by Leloir et al. (14) the structural relation between the enzyme and polysaccharide in the starch synthetase reaction system is similar to the conditions existing in whole grains. The colloidal condition of the reaction system was not maintained, however, when the 2 enzymes were combined. This resulted in a heterogeneous mixture consisting mainly of the liquid phase. Therefore, the over-all mechanism of sucrose-starch conversion in vivo also may be more efficient than the results obtained in a test tube.

Leloir's group (14,16) established that the molecular structure of polysaccharides synthesized by both glycogen and starch synthetase is the α-(1–4) glucosidic type, thus the enzyme is often called amylose synthetase (36). Nelson and Rines (23) recently reported, however, the deficiency of starch synthetase in waxy corn and proposed a possible regulation mechanism in the biosynthesis of amylose and amylpectin. Although we have not examined this view with waxy and nonwaxy rice grains, the results in table II show a measurable incorporation of glucose-C14 from UDPG to both the amylose and amylpectin fractions. These results are not easily explained by the observation of Nelson and Rines.

The important role of phosphorylase in starch synthesis cannot be neglected as has been discussed by Badenhuizen (5) and Porter (26), and more work is needed to clarify its role in the starch formation in the developing rice grains.

Summary

Starch granules catalyzing starch synthesis were prepared from ripening rice grains. Starch synthesis was confirmed by both the enzymic assay for uridine diphosphate liberated during the transglucosylase reaction and glucose-C14 transfer to starch molecules from uridine diphosphate glucose-C14. Glucose-C14 was incorporated into both the amylose and amylpectin fractions of the starch molecules.

A measurable amount of glucose-C14 was transferred from sucrose-C14 to starch in the presence of starch granules, sucrose synthetase and uridine diphosphate, indicating a possible operation in vivo of the reversal of sucrose synthesis to provide uridine diphosphate glucose. Glucose-C14 was incorporated from sucrose into both the amylose and amylpectin fractions of the starch molecules in much the same ratio as that obtained from uridine diphosphate glucose-C14.

A crude enzyme preparation catalyzing sucrose synthesis was also found to have uridine diphosphate glucose pyrophosphorylase and uridine diphosphate phosphokinase activities, indicating the existence in rice grains of a mechanism for uridine diphosphate glucose formation from glucose-1-phosphate. The
crude rice grain extract also exhibited strong hexokinase activity. Fructose-6-phosphate, glucose-6-phosphate, and glucose-1-phosphate were formed when sucrose utilization was combined with the hexokinase reaction.

Literature Cited